Kinetics of glucose transport and sequestration in lactating bovine mammary glands measured in vivo with a paired indicator/nutrient dilution technique

Qiao, Fulong, Donald R. Trout, Changting Xiao, and John P. Cant. Kinetics of glucose transport and sequestration in lactating bovine mammary glands measured in vivo with a paired indicator/nutrient dilution technique. J Appl Physiol 99: 799–806, 2005. First published May 19, 2005; doi:10.1152/japplphysiol.00386.2004.—To quantify kinetics of mammary glucose utilization in vivo, 24 paired glucose and extracellular indicator (p-aminobenzoic acid) dilution curves across intact bovine mammary glands were obtained after bolus injections into the external iliac artery. Dilution curves were analyzed using a compartmental capillary, convolution integration model. Four candidate submodels of glucose transport and metabolism in capillary supply zones were fit to the glucose dilution curves and evaluated. Model I, with one extracellular compartment for glucose and first-order unidirectional uptake, failed, indicating that efflux of glucose from the intracellular space could not be ignored. Model II, with first-order exchanges between extracellular and intracellular compartments and sequestration from the latter, was overdefined because unidirectional clearance of glucose was at least five times the blood flow rate and 20 times the net clearance rate. Model III, combining extracellular and intracellular space into one compartment, was superior in its goodness-of-fit to curves and identifiability of parameters. Michaelis-Menten parameters of sequestration were not identifiable. Parameters of the optimal compartmental capillary, convolution integration model were applicable to both the dynamics of injected glucose dilution and the steady-state background arteriovenous difference of glucose. Glucose sequestration followed first-order kinetics between 0 and 7 mM extracellular glucose with an average rate constant of 0.006 s⁻¹ or a clearance of 44 ml/s. The ratio of intracellular to extracellular glucose distribution space was 0.34, which is considerably lower than the expected intracellular volume and suggests an intracellular occlusion compartment with which extracellular glucose rapidly exchanges.

lactose synthesis; glucose uptake; arteriovenous differences

NET UPTAKE OF GLUCOSE by the mammary glands of a lactating cow consumes 60 to 70% of the whole body glucose turnover and up to 90% of that uptake is used for lactose synthesis (1, 18). As a major osmolyte in milk, lactose concentration changes little and lactose synthesis rate is the main determinant of overall milk yield (13). Glucose is also metabolized in mammary tissue to facilitate synthesis of other milk components such as fatty acids (14). Thus the control of glucose transport and metabolism by mammary glands is important to milk synthesis and the glucose economy of the lactating animal.

Infusion of glucose for 10 h into the arterial supply of bovine mammary glands to increase blood plasma concentration from 3.6 to 6.4 mM caused only an 18% increase in milk lactose secretion rate (5). Similarly, lactose synthesis rate was unrelated to circulating glucose concentration between 4.0 and 5.0 mM but it varied 25% at a given glucose concentration (4). Throughout a 40-wk lactation, the typical monoexponential decline in milk yield of 1 to 2% per week (19) results in a halving of lactose synthesis rate. These observations suggest that lactose synthesis in the cow responds not to circulating concentrations of glucose but to a centrally dictated mammary setpoint. Kinetically, the insensitivity to glucose concentration suggests that lactose synthesis operates close to its Vmax, meaning the Kₘ is very low relative to physiological glucose concentration. However, the Kₘ of lactose synthesis by cultured bovine mammary epithelial cells was 3.5 and 4.7 mM (8, 24) and the Kₘ of 3-O-methylglucose transport in vivo was 8 mM (23), whereas normal extracellular mammary glucose is 1.5 to 3.5 mM. The in vitro kinetics of mammary lactose synthesis appear to contradict the lack of sensitivity in vivo.

The balance of mammary glucose uptake not sequestered in lactose is oxidized. In the 10-h arterial glucose infusion experiment, where lactose synthesis only increased 18%, net uptake of glucose by the mammary glands increased 39% (5), indicating responsiveness to substrate concentration in the glucose oxidation pathways. Again, the opposite is evident from in vitro experiments in which the Kₘ for glucose oxidation was 1.1 mM (7, 24).

Although kinetics of metabolic transformations are readily measured in vitro, they are not quantitatively applicable to in vivo rates of metabolism. In the current study, to resolve the apparent contradiction between in vitro and in vivo responses to glucose, a paired indicator/nutrient dilution technique was implemented to measure glucose transport and metabolism kinetics in intact lactating bovine mammary glands in vivo. Para-aminobenzoic acid (PAH) was used as the extracellular indicator and D-glucose as the nutrient of interest. Venous PAH and glucose dilution curves were analyzed using the novel compartmental capillary, convolution integration (CCCI) model described in a companion paper (17). In the CCCI model, all capillaries are identical and consist of well-mixed compartments representing extracellular and intracellular spaces and the various fluxes between and without. The heterogeneity of nonexchanging vessel transit times is used to transform concentrations of glucose in each single capillary outflow, cₓ_v(t) (mM), to concentrations in the whole organ outflow, c(t) (mM), in a linear accumulative manner. A normal distribution function, f(t), describes the density distri-
bution of nonexchanging vessel transit times with mean \( \bar{t}_v \) (s) and standard deviation \( \sigma \) (s) obtained from the PAH curve (17). These parameters of the mammary extracellular space were presented by Qiao et al. (17). The concentration of glucose in the organ outflow is then a convolution of \( f(t) \) with \( c_{sc,v}(t) \),

\[
c_s(t) = \int_0^t c_{sc,v}(t - \tau)f(\tau)d\tau
\]

where \( \tau \) is the dummy variable of integration. This formulation of the outflow problem allows transport and sequestration of glucose within a single capillary to be described with relative ease by a compartmental submodel. In this paper, we tested several candidate submodels for their ability to describe dynamics of glucose exchange during a single pass through the mammary glands with the objectives of evaluating the CCCI model in a paired indicator/nutrient dilution experiment, selecting the best submodel of mammary glucose dynamics, and quantifying kinetics of glucose transport and metabolism in intact bovine mammary glands in vivo.

**Table 1. Flux equations for candidate submodels I–IV of glucose transport and metabolism in mammary capillary supply zones**

<table>
<thead>
<tr>
<th>Submodel</th>
<th>( j_1(t) )</th>
<th>( j_2(t) )</th>
<th>( j_3(t) )</th>
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<tr>
<td>I</td>
<td>( k_1 V_e c_{sc,v}(t) )</td>
<td>0</td>
<td>0</td>
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<tr>
<td>II</td>
<td>( k_1 V_e c_{sc,v}(t) )</td>
<td>( k_2 V_e c_{sc,v}(t) )</td>
<td>( k_3 V_e c_{sc,v}(t) )</td>
</tr>
<tr>
<td>III</td>
<td>instantaneous</td>
<td>instantaneous</td>
<td>( k_3(V_e + V_c) c_{sc,v}(t) )</td>
</tr>
<tr>
<td>IV</td>
<td>instantaneous</td>
<td>instantaneous</td>
<td>( V_{in}(V_e + V_c) c_{sc,v}(t) )</td>
</tr>
</tbody>
</table>

Values are in mmol/s; \( j_1(t) \), \( j_2(t) \), and \( j_3(t) \) are velocities of influx, efflux, and sequestration, respectively, as depicted in Fig. 1. Equation structures are discussed in the text.

**MATERIALS AND METHODS**

A total of 33 paired PAH and glucose bolus injections into the external iliac artery of four lactating cows was conducted as described previously (17). The PAH dose was 0.06 to 0.1 g, and the glucose dose ranged from 1.1 to 22.2 mmol. Doses were administered over a period of 1 to 2 s. Glucose concentrations in each of 25 venous samples collected at 5-s intervals from the start of injection and a background arterial sample were analyzed by the glucose oxidase method (Sigma Diagnostics Procedure No. 510, Sigma-Aldrich Canada, Oakville, Ontario, Canada).

Candidate submodels of glucose dynamics in the single capillary were based on two compartments to represent extracellular and intracellular distribution spaces (Fig. 1). Sequestration of glucose occurs from the intracellular compartment. After conservation of mass, the general differential equations to describe the extracellular and intracellular concentration functions \( c_{sc,v}(t) \) and \( c_{in}(t) \), respectively, were

\[
\begin{align*}
\frac{dc_{sc,v}(t)}{dt} & = \left[ \frac{q_0}{F \cdot t_{dose}} p(t) + c_d(0) \right] F - j_1(t) - j_2(t) - j_3(t) \frac{1}{V_e} \\
\frac{dc_{in}(t)}{dt} & = j_1(t) - j_2(t) - j_3(t) \frac{1}{V_e}
\end{align*}
\]

where \( q_0 \) is the dose injected (mmol), \( F \) is the blood flow rate (l/s), \( t_{dose} \) is the noninstantaneous duration of the injection (s), \( p(t) \) is a rectangular pulse equal to 1 between \( t_0 \) and \( t_{dose} \) and 0 otherwise, \( c_d(0) \) is the background arterial concentration of glucose, \( c_{sc,v}(t) \) is the venous outflow concentration, \( F \) is the blood flow rate, and \( j_1(t) \), \( j_2(t) \), and \( j_3(t) \) are velocities of influx, efflux, and sequestration, respectively.

Fig. 1. Schematic illustration of candidate single-capillary glucose submodels within the compartmental capillary, convolution integration (CCCI) model; \( q_0 \) is the dose of glucose injected into the artery, \( t_{dose} \) is the duration of injection, \( p(t) \) is a rectangular pulse equal to 1 between \( t_0 \) and \( t_{dose} \) and 0 otherwise, \( c_d(0) \) is the background arterial concentration of glucose, \( c_{sc,v}(t) \) is the venous outflow concentration, \( F \) is the blood flow rate, and \( j_1(t) \), \( j_2(t) \), and \( j_3(t) \) are velocities of influx, efflux, and sequestration, respectively.
arteriovenous differences. The $k_1$ at steady state, before injection of the dose, was calculated by setting Eq. 2 to 0 to yield

$$k_{1_{bg}} = \frac{c_v(0) - c_c(0)}{c_c(0)} b$$

(3)

The $k_1$ estimated from glucose dilution curves should match the $k_{1_{bg}}$. Otherwise, model I is considered to have failed.

In model II, an intracellular compartment is considered in addition to the extracellular one. Both influx $j_1(t)$ and efflux $j_2(t)$ between these two compartments are assumed to follow identical first-order kinetics where $k_1 V_v = k_2 V_c$. Rate of glucose metabolism also follows first-order kinetics. The transport parameter $k_1$ (s$^{-1}$), the ratio of intracellular to extracellular distribution volumes $r_v$, and the metabolism rate constant $k_3$ (s$^{-1}$) were estimated by fitting glucose enrichment curves. The basal intracellular glucose concentration $c_c(0)$ (mM) was calculated from the fitted $k_1$ value based on the fact that, at steady state, the net uptake of glucose across the mammary glands can be defined as either the balance between arterial and venous flows $c_c(0) F - c_c(0) F$ or the balance between extracellular and intracellular compartments $k_1 V_v c_v(0) - k_2 V_c c_c(0)$. Where $k_1 V_v = k_2 V_c$, the equality of these two definitions of net uptake yields, upon rearranging,

$$c_c(0) = c_c(0) - \frac{c_v(0) - c_c(0)}{k_1} b$$

(4)

where $k_1 > 0$. The basal metabolism rate constant is then

$$k_{3_{bg}} = \frac{k_3 [c_c(0) - c_v(0)]}{c_c(0) r_v}$$

(5)

Again, the relationship between $k_{3_{bg}}$ and $k_3$ is a test of the model’s ability to describe glucose uptake.

Model III is a special case of model II in which $k_1$ and $k_3$ are high enough to ignore the barrier between extracellular and intracellular compartments and combine these two compartments into one. It differs from model I in that an intracellular compartment is considered. The parameters $k_1$ and $r_v$ were estimated from glucose dilution curves. The steady-state glucose metabolism rate constant is

$$k_{3_{bg}} = \left(\frac{c_c(0) - c_v(0)}{c_c(0)}\right) b \frac{1}{1 + r_v}$$

(6)

Model IV is the same as model III except that glucose metabolism follows Michaelis-Menten kinetics rather than first-order kinetics. To satisfy both the enrichment curve and the basal arteriovenous difference of glucose, the parameters $K_m$ (mM) and $r_v$ were estimated by fitting the glucose curves and $V_{max}$ (mM s$^{-1}$) was calculated from the basal arteriovenous difference constraint as

$$V_{max} = \left[\frac{c_c(0) - c_v(0)}{c_c(0)}\right] \left[\frac{r_v}{K_m + r_v}\right]$$

(7)

In addition to the model validation criteria specified above, each model was evaluated for goodness-of-fit to observations, randomness of residuals on the time course, and identifiability and plausibility of parameters.

**RESULTS**

The ratio of glucose to PAH in the injectates ($r$) and basal glucose concentrations in the artery and vein are listed in Table 2. The $r$ and iA values were used to represent the exact glucose dose delivered to the mammary glands.

Thirty-three paired PAH/glucose curves were obtained from four lactating cows. A typical set of curves is shown in Fig. 2. Low doses (<1 g) of injected glucose did not result in sufficient elevation of venous glucose over background to be precisely detected by the enzymatic assay, so nine sets of curves (from cows 2410 and 382) were not analyzed. Least-squares estimates of parameters of the four tentative glucose
transport and metabolism models (Table 1) are summarized in Table 3 and final fits to selected glucose dilution curves are shown in Fig. 3.

The goodness-of-fit of each model was evaluated from both $r^2$ and the square root of the mean square prediction error (rMSPE). Using Tukey’s multiple range test, the $r^2$ of model I (Table 3) was significantly lower ($P<0.001$) than those of the other models, and its rMSPE was significantly higher ($P<0.001$). There were no significant differences in $r^2$ and rMSPE between models II, III, and IV or between cows. Because models I, II, and III are nested, fits to each curve were compared by partial $F$-tests, which yielded, on average, $P<0.001$ for comparisons of models I and II, $P<0.001$ between models I and III, and $P=0.441$ between models III and II. Model II provided a significantly better fit than model III to 4 of the 24 glucose curves. According to the coefficient of variation of parameter estimation (approximate standard error/final estimate), only models I and III converged well and were, therefore, identifiable.

Model I did not describe the glucose dynamics as well as the other three. According to the assumption that efflux is negligible, the tail of the glucose curve must decline faster than that of the paired PAH curve because unidirectional uptake occurs in addition to simple wash out. However, the paired glucose and PAH curves actually became closer to each other with time (Fig. 2). Underestimation of tail concentrations was compensated by overestimation of peak concentrations in the least-squares parameterization. Despite the poor fits, model I converged on a $k_1$ solution, which agreed with $k_{1_bg}$ calculated from basal arteriovenous differences. The slope of the relationship between these two parameters was not different from 1.0 ($P=0.69$; Fig. 4A).

With compartments for both the extracellular and intracellular spaces, model II explained the glucose curves as well as

### Table 3. Mean, SD, and mean CVE of glucose transport and metabolism parameters estimated from least-squares fits of candidate submodels I–IV to 24 paired glucose/PAH dilution curves across the mammary glands

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>Mean±SD</th>
<th>CVE</th>
<th>Mean±SD</th>
<th>CVE</th>
<th>Mean±SD</th>
<th>CVE</th>
<th>Mean±SD</th>
<th>CVE</th>
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<tr>
<td>$k_1$, $s^{-1}$</td>
<td>I</td>
<td>0.0098±0.0016</td>
<td>0.22</td>
<td>87±143</td>
<td>428</td>
<td>0.0068±0.0014</td>
<td>0.15</td>
<td>0.36±0.12</td>
<td>0.35</td>
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<tr>
<td>$k_3$, $s^{-1}$</td>
<td>II</td>
<td>0.025±0.0084</td>
<td>11</td>
<td>0.39±0.11</td>
<td>10</td>
<td>0.34±0.087</td>
<td>0.32</td>
<td>13218±18418</td>
<td>1.4×10^6</td>
</tr>
<tr>
<td>$K_{m,M}$</td>
<td>III</td>
<td>0.39±0.11</td>
<td>10</td>
<td>0.34±0.087</td>
<td>0.32</td>
<td>13218±18418</td>
<td>1.4×10^6</td>
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<tr>
<td>$V_{max,m/s}$</td>
<td>IV</td>
<td>0.96±0.017</td>
<td>1.0±0.006</td>
<td>1.0±0.006</td>
<td>0.99±0.014</td>
<td>88±145</td>
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<tr>
<td>$r^2$</td>
<td>I</td>
<td>0.174±0.045</td>
<td>0.053±0.022</td>
<td>0.058±0.024</td>
<td>0.071±0.034</td>
<td>0.033±0.004</td>
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<tr>
<td>rMSPE, mM</td>
<td>II</td>
<td>0.033±0.004</td>
<td>0.033±0.004</td>
<td>0.033±0.004</td>
<td>0.033±0.004</td>
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<tr>
<td>$b, s^{-1}$</td>
<td>III</td>
<td>0.033±0.004</td>
<td>0.033±0.004</td>
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<td>0.033±0.004</td>
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<tr>
<td>$c_c(0)$</td>
<td>IV</td>
<td>2.99±0.42</td>
<td>0.026±0.012</td>
<td>0.0067±0.0020</td>
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CVE, coefficient of variation of estimation. To obtain the mean CVE, the approximate standard error of estimation of each parameter value, as a proportion of the parameter value, was averaged. Steady-state parameters $k_{1_bg}$, $k_{3_bg}$, and $c_c(0)$ were calculated for each fit according to Eqs. 3 to 6 using the value of $b$ from PAH curves (17).
models III and IV (Fig. 3). However, although the non-steady-state $k_3$ was correlated with $k_{3, bg}$ (Fig. 4B), the slope was different from 1.0 ($P = 0.03$). In addition, the parameter $k_1$ was not identifiable from most of the glucose curves (Table 3). Analysis of residual sums of squares indicated that there was no optimum $k_1$ value (Fig. 5). Increasing $k_1$ beyond a value of 0.5 s$^{-1}$ had little impact on the goodness-of-fit because at such high turnover, the intracellular and extracellular glucose concentrations become essentially equal. The average monoexponential washout slope $b$ from the 24 PAH curves was 0.033 s$^{-1}$ (17), whereas $k_1$ was greater than 0.5 s$^{-1}$ for nine of the curves and averaged 0.18 s$^{-1}$ for the others. Unidirectional clearance was thus at least five times the rate of plasma flow through the mammary glands. Although glucose uptake is flow limited, transport was apparently not a limiting factor for glucose utilization in the bovine mammary glands. Model II was overdefined, and it was decided to combine extracellular and intracellular spaces into one well-mixed compartment for models III and IV.

Model III predicted the glucose curve well (Fig. 3) and possessed the most identifiable parameters (Tables 3 and 4). This model overcame the limitation of model I that the intracellular glucose distribution volume and efflux from it were ignored. The relationship between steady-state and non-steady-state parameters, $k_{1, bg}$ and $k_3$, of glucose metabolic rate (Fig. 4C) had a slope not different from 1.0 ($P = 0.63$) and a non-significant intercept ($P = 0.6$). The fact that model III with essentially instantaneous transport described both injected dose...
and basal arteriovenous differences best supports the conclusion from model II that glucose transport did not limit glucose utilization in the mammary glands.

Model IV assigned Michaelis-Menten kinetics to the glucose sequestration function instead of first-order kinetics, but $K_m$ and $V_{max}$ were not well identified by many glucose curves (Table 3). Using a nonlinear approach to describe a linear system often results in multiple parameter solutions. The $k_{3_{bg}}$ estimated from model III was equivalent to the term $V_{max}/K_m$ from model IV (Fig. 4D), which indicates that glucose sequestration in the mammary gland did not follow Michaelis-Menten kinetics, but rather simple linear kinetics.

Because model III was superior to the other models in identifiability of parameters and goodness-of-fit to the observed data, further analysis focused only on model III. There was no correlation between residuals and corresponding sampling time (Fig. 6A). In addition, there was no correlation between parameter estimates and the glucose dose (Fig. 6, B and C). These results demonstrate that the indicator dilution technique yielded parameters representing instantaneous physiological status without influencing it.

**DISCUSSION**

Mammary glucose compartmentalization. The failure of model I suggested that efflux of injected glucose from within cells had to be considered to fit the venous dilution curves. The primary glucose transporter in lactating mammary glands is GLUT1, the bidirectional facilitative and insulin-insensitive transporter of erythrocytes (2, 3, 28). Although the exact mechanism of GLUT1 function remains to be elucidated, it is clear from the time course of uptake by erythrocytes that the transporter translocates extracellular sugar across the plasma membrane into an intracellular compartment from which the sugar may diffuse into the bulk cytosol or, with a greater likelihood, escape back to the extracellular space (12, 16). The same behavior was observed in bovine mammary epithelial cells in vitro (25) and is now apparent from in vivo mammary dilution curves.

Parameters for model II indicated that the unidirectional clearance of glucose by the mammary glands ($k_1V_e$) was at least five times the plasma flow rate and 20 times the net

<table>
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<tr>
<th>Cow</th>
<th>Curve</th>
<th>$k_3$ ($s^{-1}$)</th>
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<th>$r_3$</th>
<th>$r_{MSPE}$, mM</th>
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$k_3$, Sequestration rate constant; $r_v$, ratio of intracellular to extracellular volume; $k_{3_{bg}}$, background glucose sequestration rate constant.

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Fig. 6. Evaluation of predictions of glucose concentration by submodel III in the CCCI model. A: residuals plotted against time for the 24 glucose dilution curves. $B$: regression between the least-squares $k_3$ value and dose of glucose acid injected. $C$: regression between the least-squares $r_v$ value and dose of glucose injected. Relationships between dose and parameter values were not significant.
clearance ($k_3 \cdot V_e$). Similarly, Goresky et al. (10) found from modeling of multiple indicator dilution across the liver in vivo that the capacity for influx of galactose into liver cells was 40 times that for galactose sequestration by phosphorylation. In human erythrocytes in vitro, the rate of sugar exchange between the extracellular and intermediate intracellular compartments was 23 times faster than the rate of exchange between the intermediate compartment and the bulk cytosol (12). Occlusion of glucose in an intermediate compartment did not appear to prevent phosphorylation in bovine mammary epithelial cells in vitro (24) but glucose utilization rate at 3.0 mM glucose was 62 nmol·h⁻¹·mg protein⁻¹, which, assuming 286 g mammary protein (22), represents a clearance of 1.6 ml/s. The clearance from extracellular to occlusion compartments was 6.5 ml/s, or four times the lactose synthesis rate. Thus, although the parameters of exchange with intracellular space in model II were difficult to estimate, the conclusion that translocation of glucose into mammary cells is many times faster than its rate of metabolism is supported by previous observations. Model III, which was superior in identifiability, assumed instantaneous transport.

The estimated ratio $r_v$ of intracellular glucose distribution volume to extracellular volume for model III was 0.34 ± 0.09. There were no significant differences between cows in $r_v$. The value of $r_v$ is low compared with an intracellular-to-extracellular water ratio of 1.5 for the whole body (21), 2.8 for the heart (20), and 4.7 for the liver (26). The extracellular volume of a 22-kg udder was estimated at 5.5 liters from the PAH dilution curves (17). Assuming 75% of udder mass is water leaves 11.0 liters of intracellular water, which is two times the extracellular volume. The estimated $r_v$ for glucose of 0.34 is thus only 17% of the expected ratio and likely represents the size of the intermediate occlusion compartment. Occlusion volume was estimated at 30–40% of intracellular glucose space in human erythrocytes (6, 12) and 35% in cultured bovine mammary epithelial cells (25). Intracellular ATP will influence the size of the occlusion space, possibly through allosteric interaction with the GLUT1 homotetramer. Occlusion volume was 40% of intracellular in erythrocyte ghosts containing 4 mM ATP and 20% in its absence (12). The glucose dilution curves are consistent with the presence in vivo of an occlusion compartment, smaller than the total cytosolic volume, with which extracellular glucose rapidly exchanges and from which glucose is relatively slowly metabolized.

**Kinetics of mammary glucose sequestration.** The modeling suggested linear kinetics of glucose sequestration between 0 and peak extracellular concentrations of 7 mM glucose. Although $K_m$ and $V_{max}$ are difficult to estimate from a time course curve even when saturation kinetics are in effect, one would not expect, with saturation, $k_{3bg}$ representing sequestration between 0 and 3 mM glucose to match $k_3$ representing sequestration above 3 mM. For example, from the in vitro data of Xiao and Cant (24) where the $K_m$ of glucose utilization by mammary epithelial cells was 4.2 mM, the glucose sequestration constant between 0 and 3 mM glucose, assuming 286 g mammary protein, was 1.6 ml/s and that between 3 and 5 mM was reduced to 1.0 ml/s. The lack of difference in vivo suggests a $K_m$ for glucose utilization greater than the in vitro $K_m$ of 4.2 mM. Multiplying the average $k_3$ value of 0.006 s⁻¹ by the compartment volume of (1 + $r_v$) 5.5 liters gives an in vivo clearance of 44 ml/s, which is 28 times the in vitro rate.

A primary objective of obtaining the glucose dilution curves was to reconcile kinetics of mammary glucose utilization in vitro with rates measured at steady state in vivo. Rates of glucose sequestration and secretion of lactose in milk are not correlated with circulating glucose concentrations (4, 7, 15), suggesting the $K_m$ for glucose utilization is low. However, $K_m$ values for transmembrane glucose transport and lactose synthesis from extracellular glucose estimated in isolated mammary epithelial cells in vitro were higher than normal extracellular glucose concentrations (8, 23, 24). In general, the in vivo kinetics of glucose fluxes in the mammary glands, estimated from the paired PAH/glucose dilution curves, support observations in vitro that there is an occlusion space for rapid, bidirectional translocation of glucose across the plasma membrane of cells and that the $K_m$ for intracellular sequestration is higher than the normal concentration of extracellular glucose. The apparently low $K_m$ implicated in correlation analysis of point estimates of plasma glucose concentration and mammary uptake could be due to control of sequestration efficiency. In a previous experiment, elevation of mammary venous glucose concentration, representing extracellular concentration, from 2.9 to 5.2 mM by infusion of 90 g/h glucose into the external iliac artery resulted in a 39% increase in net glucose uptake and an 18% increase in incorporation into lactose (5). At a constant clearance rate across glucose concentrations, one would expect net glucose to have increased to the same degree as venous glucose concentration, i.e., 80%. That it only increased 39% suggests a downregulation of glucose sequestration capacity during the 10 h of glucose infusion.

Sequestration of free glucose in mammary epithelial cells is accomplished in two ways: phosphorylation by hexokinase (EC 2.7.1.1) or hexosylation by lactose synthase (EC 2.4.1.22). Approximately 70% of the net glucose uptake from blood by lactating bovine mammary glands is used for lactose synthesis and the remainder is oxidized in pentose and TCA cycles (14). The lactose is synthesized in the Golgi apparatus from one free glucose and one uridine diphosphate galactose arising from glucose-6-phosphate. Thus about 65% of net glucose uptake is phosphorylated by hexokinase. Xiao and Cant (24), applying the methods of metabolic control analysis, estimated that hexokinase exerted 80% of the control over the flux of extracellular glucose to lactose in vitro and lactose synthase exerted <1%. Downregulation of glucose sequestration capacity of the mammary glands is therefore likely to be primarily due to hexokinase activity.

The linear kinetics observed here, where $k_3$ for an exogenous dose was equal to $k_{3bg}$, allow net clearance to be calculated from a single steady-state measurement of arteriovenous difference and blood flow according to Eq. 6, first used by Hanigan et al. (11), as

$$k_3 V_e (1 + r_v) = \frac{[c_i(0) - c_e(0)]F}{c_i(0)}$$

In the 10-h arterial infusion experiment of Cant et al. (5), mammary glucose clearance during saline infusion, calculated according to Eq. 8, was 58 ml/s and, in the last 3 h of glucose administration, clearance was 44 ml/s. Blood flow rate was reduced on the glucose treatment, which may have contributed to the decreased clearance; $V_e$ and $r_v$ were not measured. From a number of point measures of mammary arteriovenous differ-
ences and blood flows, Hanigan et al. (11) calculated an average glucose clearance of 40 ml/s. The 95% confidence interval around the estimate was ±8.4 ml/s, indicating a large variation in kinetic parameters for a narrow range of extracellular glucose concentrations. Regulation of sequestration capacity is implicated. Periodic measures of glucose arteriovenous differences and blood flows throughout lactation in sheep (7) yield clearance estimates of 5.1 ml/s at peak lactation, declining to 2.3 ml/s when milk yield had fallen by 44%. The conclusion to be drawn from these observations is that, at any one instant, the kinetics of glucose sequestration by mammary glands are essentially linear between 0 and 7 mM glucose, but the long-term consequence of up- and downregulation of sequestration capacity is a lack of relationship between glucose concentration and net glucose uptake.

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